

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/557</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/02165</b> <b>(43) International Publication Date:</b> 21 January 1999 (21.01.99)
<b>(21) International Application Number:</b> PCT/SE98/01368 <b>(22) International Filing Date:</b> 10 July 1998 (10.07.98)  <b>(30) Priority Data:</b> 9702706-4 11 July 1997 (11.07.97) SE  <b>(71) Applicant (for all designated States except US):</b> PHARMACIA & UPJOHN AB [SE/SE]; S-112 87 Stockholm (SE).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> STJERNSCHANTZ, Johan [FI/SE]; Villavägen 1 B, S-752 38 Uppsala (SE). RESUL, Bahram [SE/SE]; Vitkålsgratan 112, S-754 49 Uppsala (SE). LAKE, Staffan [SE/SE]; Farkostvägen 8, S-181 35 Lidingö (SE).  <b>(74) Agents:</b> SVANSTRÖM, Pär et al.; Pharmacia & Upjohn AB, S-751 82 Uppsala (SE).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PROSTAGLANDIN DERIVATIVES DEVOID OF SIDE-EFFECTS FOR THE TREATMENT OF GLAUCOMA  <b>(57) Abstract</b>  A new method and compositions for the treatment of glaucoma and ocular hypertension are described. The method is based on the usage of EP <sub>1</sub> prostanoid receptor agonists which effectively reduce the intraocular pressure but have no, or reduced effect on iris pigmentation. The prostaglandin analogue which is an EP <sub>1</sub> selective agonist is applied topically on the eye.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## **PROSTAGLANDIN DERIVATIVES DEVOID OF SIDE-EFFECTS FOR THE TREATMENT OF GLAUCOMA**

The invention is related to a method of treating glaucoma and ocular hypertension utilizing prostaglandin analogues or derivatives that are devoid of, or have reduced melanogenic effect in the eye. The invention also relates to ophthalmic compositions containing prostaglandin compounds devoid of, or have reduced melanogenic effect in the eye.

Glaucoma is an eye disorder characterized by increased intraocular pressure, excavation of the optic nerve head, and gradual loss of the visual field. An abnormally high intraocular pressure is commonly known to be detrimental to the eye, and there are clear indications that in glaucoma the intraocular pressure is the most important factor causing degenerative changes in the retina and the optic nerve head. The exact pathophysiological mechanism of open angle glaucoma is, however, still unknown. Unless treated, glaucoma may lead to blindness, the course of the disease typically being slow with progressive loss of vision.

The intraocular pressure (IOP) can be defined according to the formula (1):

$$(1) \quad IOP = P_e + (F_t - F_u) \times R$$

where  $P_e$  is the episcleral venous pressure,  $F_t$  the formation of aqueous humor,  $F_u$  the part of the aqueous humor which exits the eye through the uveoscleral outflow pathway, and  $R$  is the resistance in the trabecular outflow pathway. The aqueous humor in the anterior and posterior chambers of the eye is produced by the ciliary processes behind the iris. It then flows through the pupil into the anterior chamber, and normally exits the eye through the trabecular meshwork and Schlemm's canal into the episcleral veins outside the eye globe. However, part of the aqueous humor may leave the eye through the uveoscleral outflow route. The flow in this route is regarded as only minimally influenced by the intraocular pressure (Bill, 1975).

The intraocular pressure in humans is normally in the range of 12-22 mmHg. At higher pressures, e.g. above 22 mmHg, there is an increased risk that the eye may be

damaged. In one particular form of glaucoma, normal tension glaucoma, damage may, however, occur at intraocular pressure levels that are within the normal physiological range. The opposite situation is also known, i.e. some individuals may exhibit an abnormally high intraocular pressure without any manifest defects in the visual field or the optic nerve head. Such conditions usually are referred to as ocular hypertension.

Glaucoma treatment can be given by means of drugs, laser or surgery. In drug treatment the purpose is to reduce either the formation of aqueous humor (Ft), or the resistance to outflow of aqueous humor (R), which according to formula (1) above will result in reduced intraocular pressure; alternatively to increase the outflow of aqueous humor through the uveoscleral route which according to formula (1) also reduces the intraocular pressure.

Prostaglandins and typically  $\text{PGF}_{2\alpha}$ , its esters and analogues, reduce the intraocular pressure mainly by increasing the uveoscleral outflow of aqueous humor (Crawford and Kaufman, 1987; Nilsson et al., 1989; Toris et al., 1993; Stjernschantz et al., 1995). The use of prostaglandins and their derivatives is described in several patents and patent applications, for instance in US 4,599,353 (Bito), US 4,952,581 (Bito), WO89/03384 (Resul and Stjernschantz), EP 170258 (Cooper), EP 253094 (Goh), and in EP 308135 (Ueno).

Prostaglandins are fatty acids usually derived from the precursors eicosatrienoic, eicosatetraenoic and eicosapentanoic acid through metabolic steps involving oxygenation. Naturally occurring prostaglandins typically have the general structure shown in Fig. 1.

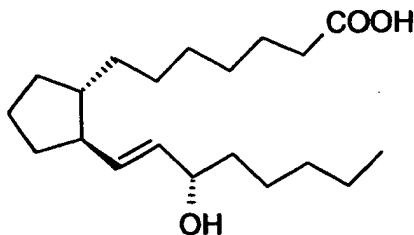


Fig. 1

The prostaglandins accordingly carry a cyclopentane ring to which two carbon chains link, the upper chain usually being called the alpha chain and the lower chain usually being called the omega chain. The prostaglandins are classified in subgroups A, B, C, D, E, F, G, H, I and J depending on the structure and substituents in the cyclopentane ring as shown in Fig. 2.

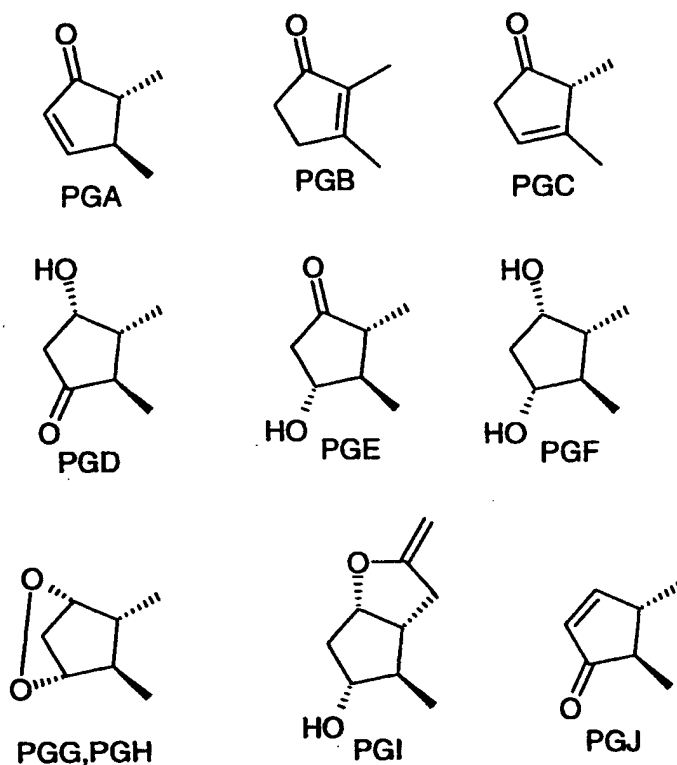


Fig. 2

The alpha chain is a 7 carbon carboxy-terminated aliphatic chain whereas the omega chain is an 8 carbon methyl-terminated aliphatic chain. Depending on the number of double bonds in these chains subscripts of 1 to 3 are given. In prostaglandins with subscript 1, e.g.  $\text{PGF}_{1\alpha}$ , the double bond is situated between carbons 13 and 14 in the omega chain, and it exhibits trans configuration in naturally occurring prostaglandins. In prostaglandins with subscript 2, e.g.  $\text{PGF}_{2\alpha}$ , an additional double bond in the cis configuration is situated between carbons 5 and 6 in the alpha chain, and finally in

prostaglandins with subscript 3, a third double bond is situated between carbons 17 and 18 in the omega chain. This double bond also exhibits cis configuration in naturally occurring prostaglandins. All naturally occurring prostaglandins carry a hydroxyl group on carbon 15, which is essential for biological activity.

The receptor system for the naturally occurring prostaglandins has only recently been elucidated. Thus most of the prostaglandin receptors have been pharmacologically characterized and their molecular structure identified by molecular biological techniques (Coleman et al., 1994). There are specific receptors for the naturally occurring prostaglandins. The receptors for PGD, PGE, PGF, PGI<sub>2</sub> (prostacyclin) and TxA<sub>2</sub> (thromboxane) being abbreviated DP, EP, FP, IP, and TP, respectively. Furthermore it has been shown that the EP receptor can be subdivided into four receptors, namely the EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, and EP<sub>4</sub> receptors. Specific tissues or cells may express only a few or many of these receptors, depending on the evolutionary development of this autacoid system in different species. Thus for instance it has been shown that the cat iris sphincter muscle expresses predominantly FP receptors that are functionally coupled and mediate constriction of the pupil, while the corresponding smooth muscle of the bovine eye expresses EP<sub>1</sub> and TP receptors, activation of either of which will result in contraction of the muscle. A compound which binds to a specific receptor and activates it is called an agonist while a compound that only binds to a receptor without activating it is called an antagonist.

With respect to the practical usefulness of many of the prostaglandins and their derivatives as suitable drugs for treatment of glaucoma or ocular hypertension, a limiting factor may be their property of causing increased pigmentation of the iris in the eye (Stjernschantz and Alm, 1996). Thus, the colour of the iris during chronic treatment in monkeys and in man tends to become darker, turning into brown. While this apparently has no negative medical consequences, it is a clear disadvantage from a cosmetic point of view, particularly in patients undergoing treatment only in one eye. It would thus be desirable to identify prostaglandins which effectively reduce the intraocular pressure without causing the side-effect of increased iridial pigmentation.

We have now unexpectedly found that prostaglandin derivatives and analogues which are selective agonists for the EP<sub>1</sub> subgroup of prostanoid receptors fulfil the criteria for a prostaglandin analogue which effectively reduces the intraocular pressure without causing increased production of pigment (melanogenesis) in the iris. The background of this finding is that in a study to identify the prostanoid receptor subtypes in the human iridial melanocytes we have found that these cells express in their cell membranes the FP, EP<sub>2</sub>, and EP<sub>3</sub> receptors, but not the EP<sub>1</sub> and TP receptors. Furthermore, we have investigated the intraocular pressure reducing effect of several relatively selective EP<sub>1</sub> agonists and found that these prostaglandin analogues effectively and potently reduce the intraocular pressure both in cats and monkeys.

Therefore, it is now apparent that, by using selective EP<sub>1</sub> receptor agonists, the intraocular pressure can be reduced in primates, and thus also in man, without increased, or with significantly reduced melanogenesis as side-effect, since the melanin producing cells, the melanocytes, lack the specific receptor necessary for the transmembrane signalling into the cell. While we at present have no clinical evidence that such selective EP<sub>1</sub> agonists do not cause increased pigmentation of the iris, since the induction time for this phenomenon to occur often is 6-12 months, and thus extremely long and costly experiments have to be carried out in primates, we can nevertheless conclude from relevant *in vitro* studies that such increased pigmentation would not occur in the absence of the specific signalling receptor in the cell membrane of the melanocytes.

Accordingly, high selectivity or specificity to the EP<sub>1</sub> receptor compared to other prostaglandin receptors in the eye characterizes the compounds to be used in the method or compositions according to the present invention. It need not to be said that the more selective the compound is for the EP<sub>1</sub> receptor, the better results are obtained but a certain advantage is of course achieved also in cases of some interaction with other receptors. High selectivity in this connection means that the effect on the EP<sub>1</sub> receptor is a least more than 5 times, especially more than 10 times, and in particular more than 100 or 1000 times the effect on the other prostaglandin receptors.

The specific prostaglandin analogues that we have used for exemplifying and proving this invention were PGF<sub>2β</sub> (1), PGF<sub>2β</sub> isopropyl ester (2), 17-phenyl-18,19,20-trinor-PGE<sub>2</sub> (3), 17-phenyl-18,19,20-trinor-PGE<sub>2</sub> isopropyl ester (4), 15(R,S)-16,16-trimethylene-PGE<sub>2</sub> (5), 15(R,S)-16,16-trimethylene-PGE<sub>2</sub> methyl ester (6), and 13,14-dihydro-17-(3-fluorophenyl)-18, 19, 20 trinor-PGE<sub>2</sub>-isopropyl ester (7). All these analogues are relatively selective EP<sub>1</sub> receptor agonists. The receptor profiles of the test compounds are presented in Table I.

**Table I.** Receptor profile of prostaglandin analogues tested (EC-50 values expressed as moles/l in functional receptor assays).

Prostaglandin	FP	EP <sub>1</sub>	EP <sub>2</sub>	EP <sub>3</sub>	DP/IP	TP
1	5x10 <sup>-6</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	>10 <sup>-4</sup>	>10 <sup>-3</sup>	
>10 <sup>-3</sup>						
3	10 <sup>-7</sup>	2x10 <sup>-8</sup>	>10 <sup>-4</sup>	>10 <sup>-4</sup>	>10 <sup>-4</sup>	
>10 <sup>-4</sup>						
5	2x10 <sup>-5</sup>	6x10 <sup>-9</sup>	2x10 <sup>-7</sup>	3x10 <sup>-8</sup> #	>10 <sup>-4</sup>	>10 <sup>-4</sup>
7	6x10 <sup>-7</sup>	4x10 <sup>-8</sup>	5x10 <sup>-5</sup>	10 <sup>-6</sup> #	>10 <sup>-4</sup>	>10 <sup>-4</sup>

# estimated based on difference in receptor assay between guinea pig vas deferens and chick ileum.

In one aspect, the invention relates to the use of selective prostaglandin EP<sub>1</sub> receptor agonists devoid of melanogenic effect for the treatment of glaucoma or ocular hypertension. The method for treating glaucoma or ocular hypertension comprises contacting the surface of the eye with an effective intraocular pressure reducing amount of a composition, containing an EP<sub>1</sub> selective prostaglandin as aforesaid, in order to reduce the intraocular pressure and to maintain said pressure at a reduced level. The composition usually contains about 0.1-100 µg, especially 1-30 µg per application of the active substance. The composition is applied topically on the eye 1-3 times daily.

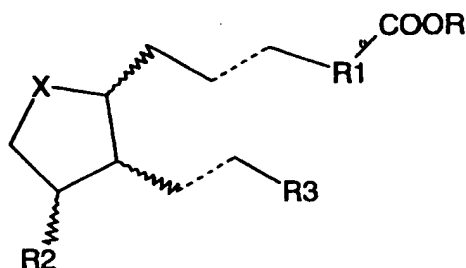


The prostaglandin derivative is mixed with an ophthalmologically compatible vehicle known per se. The vehicle which may be employed for preparing compositions of this invention comprises aqueous solutions, e.g. physiological saline, oil solutions, or ointments. The vehicle may furthermore contain ophthalmologically compatible preservatives such as e.g. benzalkonium chloride, surfactants, such as polysorbate 80, liposomes or polymers, for example methyl cellulose, polyvinyl alcohol, polyvinyl pyrrolidone and hyaluronic acid; these may be used for increasing the viscosity. Furthermore it is also possible to use soluble or insoluble drug inserts.

In another aspect, the invention relates to ophthalmological compositions for medical treatment of glaucoma or ocular hypertension which comprise an effective intraocular pressure reducing amount of a prostaglandin analogue which is a selective agonist of EP<sub>1</sub> receptors as defined above and an ophthalmologically compatible carrier. The effective amount usually comprises a dose of about 0.1-100 µg in about 10-50 µl of the composition. The compositions according to the present invention are clear improvements over the prior art prostaglandin compositions due to the selectivity of the active compound for EP<sub>1</sub> receptors compared to other prostaglandin receptors with the risk for pigmentation eliminated or at least substantially reduced.

In still another aspect, the invention relates to the use of the prostaglandin analogue for the preparation of a medicament for treatment of glaucoma and ocular hypertension.

Preferably, the prostaglandin analogue is derived from PGF or PGE type prostaglandins. Particularly, the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the  $\alpha$  or  $\beta$  configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably  $C_{1-10}$  alkyl, cycloalkyl, preferably  $C_{3-8}$  cycloalkyl, aryl, arylalkyl, preferably aryl- $C_{2-5}$  alkyl, or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably  $C_{3-7}$  cycloalkyl, cycloalkenyl, preferably  $C_{3-7}$  cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, preferably  $C_{1-10}$  alkyl, especially  $C_{1-6}$  alkyl, or a cycloalkyl, preferably  $C_{3-8}$  cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from  $C_{1-5}$  alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably  $C_{3-8}$  cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with  $C_{1-3}$  alkyl,  $C_{1-3}$  alkoxy, hydroxy, nitro, trifluoromethyl or halogen;  
or a pharmaceutically acceptable salt or ester thereof.

Aryl is preferably substituted or unsubstituted phenyl.

Exemplary heteroaryl groups are thiophene, furan, thiazole, isothiazole, oxazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine.

Aryl, heteroaryl and cycloalkyl may be mono- or independently di- or multi-substituted by C<sub>1-3</sub> alkyl, C<sub>1-3</sub> alkoxy, hydroxy, nitro, trifluoromethyl or halogen.

Unsaturated alkyl may contain one or more double and/or triple bonds.

Halogen is fluorine, chlorine, bromine or iodine, especially fluorine, chlorine or bromine.

The prostaglandins may be in epimeric mixtures as well as in the form of the individual epimers.

The invention is illustrated by means of the following non-limiting examples:

Identification of prostaglandin receptors. The prostaglandin receptors were identified using the reversed transcriptase polymerase chain reaction (RT-PCR) technique. Specific primers were designed for the FP, EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, and TP receptors. The primers used in the assays are presented in Table II. RT-PCR was performed on mRNA isolated from human iridial melanocytes in culture. The cultured cells were used for preparing mRNA. The RT-PCR mix was analysed on agarose gel and bands of expected size were cloned and sequenced. The deduced sequences were analysed for similarity to each prostaglandin receptor sequence.

Methods. Human iridial melanocytes were isolated and cultured according to Hu et al. (1993) and used in early passages. Cells were grown to confluence and harvested for the mRNA enrichment.

mRNA was isolated using Dynals mRNA Direct System (Dynal A/S, Norway) according to the manufacturer's instructions. 100.000-200.000 human melanocyte cells were used in the enrichment. mRNA is covalently bound to an oligo-dT labeled Dynabead. Using reverse transcriptase the first strand cDNA is synthesized directly on

the Dynabeads with the oligo-dT as a reverse transcriptase primer. The second strand cDNA is synthesized using a known 3' sequence primer from respective prostaglandin receptor, resulting in double stranded cDNA. The same set of Dynabeads was used for each receptor RT-PCR. Receptor specific primers were used for PCR amplifying DNA from Dynabead bound cDNA according to the manufacturer's instructions. For the FP and EP<sub>3</sub> receptor reactions the PCR was performed in 50 µl final volume with 5% DMSO, 200 µM dNTPs and 20 pmoles of each primer. For the other receptors hot start with AmpliWax pellets (Perkin Elmer, USA) was used in a final volume of 75 µl with 5% DMSO, 200 µM dNTPs and 20 pmoles of each primer.

Table II. Prostaglandin receptor specific primers.

---

FP primers:

Primary primers; CAC AAC CTG CCA GAC GGA AAA C and CCA GTC TTT  
GAT GTC TTC TGT G

Secondary primers; CAG TAA TCT TCA TGA CAG TGG G and TTG TAG AAA  
CAC CAG GTC CTG G

---

EP<sub>1</sub> primers:

primary primers; TGT GGC ATG GCC GTG GAG and ACC AAC ACC AGC ATT  
GGG C

secondary primers; CTG CAG GGA GGT AGA GCT C and GGC ACG TGG TGC  
TTC ATC G

---

**EP<sub>2</sub> primers:**

primary primers; CAA CCA TGC CTA TTT CTA CAG C and TCT CGC TCC AAA  
CTT GGC TG

secondary primers; CTA CGT GGA CAA GCG ATT GGC and TGG TTG ACG  
AAC ACT CGC AC

---

**EP<sub>3</sub> primers:**

primary primers; GGG ATC CAA GAT CTG GTT CAG and GCC TTC CCG ATC  
ACC ATG CTG

secondary primers; CGC AAG AAG TCG TTC CTG CTG and CAC CAA GTC  
CCG GGC CAC TG

---

**TP primers:**

primary primers; CTG GTG ACC GGT ACC ATC GTG GTG T and GTA GAT CTA  
CTG CAG CCC GGA GCG C

secondary primers; TCG CTA CAC CGT GCA ATA CC and GGC TGG AGG GAC  
AGC GAC

---

The PCR mix from these reactions was analyzed on a 1% LMP agarose gel (BioRad Laboratories, USA). DNA fragments of the expected size were TA-cloned using a TA-cloning kit according to the manufacturer's instructions (Invitrogen Inc., USA), and sequenced on an Applied Biosystem Model 373A DNA sequencing system (Applied Biosystems Inc., USA) according to Applied Biosystems' protocol for their Taq Dye Dioxo Terminator cycle sequencing kit. The generated primary data were processed on a VAX computer using the sequence analysis programs from Genetics Computer Group Inc., Madison, USA (Devereux, J., et al., Nucleic Acids Research 12 (1): 387-395 (1984).

**Results:** In human iridial melanocytes based on our RT-PCR we could show an expression of EP, EP<sub>2</sub> and EP<sub>3</sub> receptors. However, we were not able to show the presence of the EP<sub>1</sub> and TP receptors (Table III). As positive controls we amplified the expected EP<sub>1</sub> and TP fragments with the same primers from a human kidney

cDNA library. We enriched poly A mRNA from human iridial melanocytes isolated at two different times and performed the PCR reactions several times with identical result.

**Table III.** RT-PCR (secondary PCR primers) of human iridial melanocyte mRNA using prostaglandin receptor specific primers (see Table II).

Gene	Correct fragment size (bp)		Sequence analysis
	<u>Observed</u>	<u>Expected</u>	
FP	+	489	Identity with FP
EP <sub>1</sub>	-	397	-
EP <sub>2</sub>	+	501	Identity with EP <sub>2</sub>
EP <sub>3</sub>	+	372	Identity with EP <sub>3</sub>
TP	-	484	-

### SYNTHESIS OF PROSTAGLANDIN DERIVATIVES

The structures of the end compounds prepared in the Examples are shown in Scheme 1 provided at the end of the description.

#### Example 1: PGF<sub>2β</sub>(compound 1)

The title compound was purchased from Cayman Chemicals Company, Ann Arbor Michigan, USA.

#### Example 2: PGF<sub>2β</sub> isopropyl ester (compound 2)

DBU (163.5 mg, 1.01 mmol) was added to a stirred solution of PGF<sub>2β</sub> (Cayman Chemicals) (60 mg, 0.169 mmol) in acetone (20 ml) at 0 °C. The mixture was allowed

to warm to room temperature, when isopropyl iodide (222.6 mg, 1.34 mmol) was added dropwise. After 48 h (TLC monitoring), the mixture was diluted with ethyl acetate (40 ml), washed with brine (30 ml), citric acid 3% (2x40 ml) and sodium hydrogen carbonate 5% (2x30 ml) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel using ethyl acetate : acetone 3:1 as eluent. This afforded a colorless oil, yield 46 mg (68%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.3 (d, 6H), 1.6-1.7 (dm, 4H), 2.0-2.2 (dm, 6H), 2.3 (t, 2H), 4.0-4.1 (m, 3H), 5.0 (sept, 1H), 5.5 (m, 2H), 5.6 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 135.9, 132.2, 130.5, 128.0, 75.3, 74.8, 72.85, 67.6, 56.23, 52.25, 51.59, 42.32, 37.35, 33.44, 31.74, 29.14, 26.66, 24.79, 22.6, 21.8, 14.03.

**Example 3:** 17-Phenyl-18,19,20-trinor-PGE<sub>2</sub> (compound 3)

The title compound was purchased from Cayman Chemicals Company, Ann Arbor Michigan, USA.

**Example 4:** 17-Phenyl-18,19,20-trinor-PGE<sub>2</sub> isopropyl ester (compound 4)

DBU (43.5 mg, 0.29 mmol) in acetonitrile (1 ml) was added dropwise to a stirred solution of compound 3 (22.1 mg, 0.057 mmol) in acetonitrile (3 ml) at 0 °C. The mixture was allowed to warm to room temperature whereupon isopropyl iodide (78.0 mg, 0.46 mmol) in acetonitrile (2 ml) was added dropwise. After being stirred for 12 h (TLC monitoring), the reaction mixture was quenched with water (8 ml), the mixture was extracted with ethyl acetate (2x50ml), and the extract was washed with brine (10 ml), citric acid 3% (10 ml), and finally sodium hydrogen carbonate 5% (10 ml). After drying with anhydrous sodium sulfate, the solvent was removed in vacuo and the residual oil was chromatographed on silica gel using ethyl acetate as eluent. This afforded 230 mg of the product (69%) of the title compound as a colorless oil: *R*<sub>f</sub> = 0.516 (ethyl acetate : acetone : HOAc 1:1:0.02); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.89 (m, 3H), 1.3 (d, 6H), 2.6-2.8 (m, 2H), 4.1 (m, 2H), 5.0 (m, 1H), 5.3-5.7 (dm, 4H), 7.2 (m, 5H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 10.9, 13.9, 21.8, 22.9, 23.8, 24.49, 24.8, 25.17, 25.6, 26.68, 28.93, 30.45, 31.77, 33.90, 34.01, 34.07, 38.8, 46.22, 53.3, 54.48, 66.83, 67.62, 68.18, 71.77, 72.21, 76.35, 77.00, 77.2,

77.64, 125.93, 126.46, 128.39, 128.44, 128.79, 130.63, 130.81, 131.04, 137.79, 213.88.

**Example 5: 15RS-16,16-trimethylene-PGE<sub>2</sub> (compound 5)**

To a stirred solution of 15RS-16,16-trimethylene-PGE<sub>2</sub> methyl ester (52 mg, 0.13 mmol) in acetone (0.4 ml) and phosphate buffer pH 7 (4 ml) was added lipase VII (40 mg). The mixture was stirred at room temperature for 24 h (TLC monitoring). The mixture was quenched with ethanol (3 ml) and extracted with ethyl acetate (2x10 ml). The organic layer was washed with brine, dried (sodium sulfate), and concentrated in vacuo furnishing 46 mg of the product as an oil.

**Example 6: 15RS-16,16-trimethylene-PGE<sub>2</sub> methyl ester (compound 6)**

The synthesis of 15RS-16,16-trimethyleneprostaglandin E<sub>2</sub> (Skotnicki, S. et al. 1977) is schematically shown in Scheme 2. Bold figures in the following refer to respective structures in Scheme 2.

**Ethyl 2,2-trimethylhexanoate (9)**

To a stirred solution of N-isopropylcyclohexylamine (56.2 g, 398 mmol) in THF (400 ml) at -78 °C was added rapidly n-BuLi (159 ml, 398 mmol of 2.5 M in hexane). To the resulting solution was added dropwise ethyl cyclobutanecarboxylate (**8**) (50 g, 390 mmol) and stirred for 30 min, then warmed to 0 °C and dropped into a solution of n-butyl iodide (159 ml, 398 mmol of 2.5 mol in hexane) in DMSO (200 ml). The reaction mixture was stirred for 1 h at room temperature (TLC monitoring). The salt was removed by filtration and the filtrate was concentrated in vacuum. The residue was dissolved in hexane and washed with HCl 2%, brine and water, then dried over sodium sulfate, and evaporated in vacuo. The residual oil was distilled (49-56 °C, 1 mmHg) to give 26.5 g (37%) of the product.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.9 (t, 3H), 1.2 (t, 3H), 1.8-2.0 (dm, 5H), 2.2-2.5 (m, 3H), 4.2 (m, 2H).

**2,2-Trimethylenehexan-1-ol (10)**



To a stirred solution of ethyl 2,2-trimethylenehexanoate (**9**) (26.5 g, 144 mmol) in dry toluene (100 ml) was added dropwise DIBAL-H (206 ml, 289 mmol of 1.4 mol in toluene) at 0 °C. The resulting solution was stirred at room temperature for 3 h (TLC monitoring), and then poured into iced HCl 5%. The organic layer was separated and washed with HCl 5%, brine, dried, filtered and concentrated to give 30 g of the product as an oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.9 (t, 3H), 1.8-2.0 (dm, 5H), 2.5 (m, 1H), 3.0 (m, 1H), 3.6 (m, 2H).

#### 2,2-Trimethylenehexaldehyde (**11**)

To a solution of 2,2-trimethylenehexan-1-ol (**10**) (30 g, 210 mmol), in DME (400 ml), was added dicyclohexanecarbodiimide (DCC) (130 g, 630 mmol), DMSO (120 ml) and orthophosphoric acid (10.3 g). The mixture was stirred at room temperature for 3 h (TLC monitoring), and filtered. The filtrate was diluted with dichloromethane (300 ml), and washed with water. The organic layer was separated. The residue was removed by filtration. The filtrate was washed with brine (100 ml), dried and concentrated in vacuum. The residue was purified by column chromatography on silica gel using hexane as eluent to give the title product (17.3 g) as an oil.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.9 (t, 3H), 1.2 (t, 3H), 1.8-2.0 (dm, 5H), 8.8 (s, 1H).

#### 4,4-Trimethylene-1-octyn-3-ol (**12**)

To a solution of lithium acetylide-ethylenediamine complex (12.2 g, 132 mmol) in DMSO (10 ml) was added a solution of 2,2-trimethylenehexaldehyde (**11**) (17 g, 120 mmol) in DMSO (20 ml) at 0 °C under N<sub>2</sub>. The mixture was stirred at room temperature for 24 h (TLC monitoring) and then poured into an ice-cold HCl 2% (50 ml) and ether (50 ml). The organic layer was separated and the aqueous layer was extracted with ether (50 ml), the combined organic phases were washed with brine, dried, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate 5:1 as eluent, which gave **12** (7.6 g, 38%) as an oil.

#### E-Tributyltin-4,4-trimethylene-1-octene-3-ol (**13**)

A mixture of 4,4-trimethylene-1-octyn-3-ol (12) (5.0 g, 30 mmol), tributyltin hydride (14.6 ml, 54.2 mmol), and AIBN (30 mg) was stirred at 130 °C for 24 h (TLC monitoring). The residue was chromatographed on silicagel using hexane and hexane:ether 9:1, respectively, as eluent, to give the title compound (13) (12.54 g, 91.4%) as an oil.

E-Tributyltin-4,4-trimethylene-3-trimethylsilyloxy-1-octene (14)

To the mixture of E-tributyltin-4,4-trimethylene-1-octene-3-ol (13) (7 g, 15.3 mmol) in DMF (100 ml) was added imidazole (2.1 g, 30.6 mmol) and trimethylsilyl chloride (2.5 g, 23.0 mmol). The reaction mixture was stirred at room temperature for 1 h (TLC monitoring). The mixture was partitioned between water (200 ml) and ether (200 ml). The organic phase was dried and evaporated in vacuo. The residue was chromatographed on silica gel using hexane as eluent to give 14 (5.53 g).

11,15-bis Trimethylsilyloxy-16,16-trimethylene-5,6-didehydro-PGE<sub>2</sub> methyl ester (17)

A dry 100-ml three-necked flask was charged with copper(I)cyanide (928 mg, 10.4 mmol) and a magnetic bar. The flask was capped with a rubber septum and heated under vacuum to remove any trace of water, and cooled to 0 °C under N<sub>2</sub>. Dry THF was added and followed by methyl lithium (14 ml, 22.4 mmol of 1.6 mol in diethyl ether), via a syringe. The mixture was stirred at 0 °C for 15 min. during which the suspension became clear and homogeneous. A solution of E-tributyltin-4,4-trimethylene-3-trimethylsilyloxy-1-octene (14) (5.9 g, 11.2 mmol) in THF (10 ml) was added, via a syringe, at 0 °C and stirred at room temperature for 30 min. To the resulting solution a solution of 4-(t-butyltrimethylsilyloxy)-cyclopentenone (15) (1.7 g, 8 mmol) in THF (6 ml), trimethylsilyl chloride (4.35 g, 40 mmol) and triethylamine (8.1 g, 80 mmol) was added, at -70 °C, successively and stirred at -70 °C for another 15 min, then for 15 min at 0 °C. The mixture was partitioned between hexane (600 ml) and water (300 ml). The organic layer was separated, dried over sodium sulfate, filtered and concentrated in vacuo to give the crude silyl enol ether as a clear oil. To the stirred solution of the silyl enol ether in THF (50 ml), under N<sub>2</sub>, at -30 °C methyl lithium (7.7 ml, 12.3 mmol of 1.6 mol in diethyl ether) was added and stirred for 30 min followed by addition of a freshly prepared methyl-1-triflate-2-hexynoate (16)

(Erhardt, P.W., et al. 1987; Caldwell A. G., et al. 1979) and stirred at  $-40^{\circ}\text{C}$  for 5 min. The resulting solution was quenched with saturated aqueous ammonium chloride solution (30 ml) and extracted with ether (3x 100 ml), dried on sodium sulfate, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate (1:1) as eluent, to give a clear oil of a mixture of 15RS isomers (2.71 g, 57.3%)  $R_f = 0.36$  ( $\text{SiO}_2$ , ether : hexane 1:1).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.2 (dm, 12H), 0.8-0.9 (ms, 18H), 1.8 (m, 2H), 2.3 (m, 4H), 3.7 (s, 3H), 3.9-4.1 (dm, 2H), 5.5-5.6 (2H). The  $^1\text{H}$  NMR was also performed on the desilylated analogue, 16,16-trimethylene-5,6-didehydro-PGE<sub>2</sub> methyl ester.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.9 (t, 3H), 1.2-1.3 (m, 3H), 1.9-2.1 (m, 4H), 3.7 (s, 3H), 4.1 (m, 2H), 5.6-5.9 (dm, 2H).

11,15-bis Trimethylsilyloxy-16,16-trimethylene-PGE<sub>2</sub> methyl ester

To a stirred solution of 11,15-bis trimethylsilyloxy-16,16-trimethylene-5,6-didehydro-PGE<sub>2</sub> methyl ester (17) (500 mg, 0.8 mmol), in benzene : cyclohexane 1 : 1 (50 ml) was added Pd-BaSO<sub>4</sub> (250 mg) and quinoline (250 mg) and stirred at  $-40^{\circ}\text{C}$  under H<sub>2</sub> atmosphere for 5 h (TLC monitoring). The reaction mixture was diluted with ether and filtered through celite, and concentrated in vacuum. The residue was chromatographed on silica gel using hexane : ethyl acetate 9:1 to give 442 mg of the corresponding product.

16,16-Trimethylene-PGE<sub>2</sub> methyl ester (6)

To the solution of 11,15-bis trimethylsilyloxy-16,16-trimethylene-PGE<sub>2</sub> methyl ester (374 mg, 0.589 mmol) in THF (18 ml) was added HF 40% (3.5 ml) in THF (1 ml) at  $0^{\circ}\text{C}$ . The reaction mixture was stirred for 5 h (TLC monitoring) and then poured into a mixture of sodium hydrogen carbonate 5% (30 ml) and ethyl acetate (50 ml). The organic layer was separated and the aqueous layer was washed with ethyl acetate (2x30 ml). The organic layers were pooled and dried, on sodium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate 1:1, and ethyl acetate successively to give 6 (75 mg, 31%), as an oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.9 (t, 3H), 1.3 (t, 6H), 2.0-2.6 (mm, 9H), (dm, 5H), 3.6 (s, 3H), 4.1 (m, 2H), 5.4 (m, 2H), 5.6-5.8 (dm, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.222, 14.9, 23.7,

24.7, 25.2, 26.2, 26.5, 26.6, 26.8, 29.7, 33.4, 36.5, 44.9, 46.0, 51.6, 54.0, 54.6, 71.9, 76.7, 77.06, 77.1, 77.38, 126.5, 126.9, 127.7, 130.9, 132.5, 132.9, 133.36, 133.46, 174.15, 214.32.

Example 7: Synthesis of 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor PGE<sub>2</sub> isopropyl ester (compound 7)

The synthesis of the title compound is schematically shown in Scheme 3. Bold figures refer to respective structures in Scheme 3.

Dimethyl-(2-oxo-4-(3-fluorophenyl)butyl) phosphonate

To a stirred suspension of sodium hydride (4.17 g, 138 mmol) previously washed with n-pentane, in dry THF (250 ml) at room temperature was added dropwise a solution of dimethyl-2-oxo-propylphosphonate (23.12 g, 132.3 mmol) in THF (110 ml). The reaction mixture was stirred for 2 h, then cooled in an ice bath and treated with a solution of n-BuLi (10.2 g, 158.7 mmol) in hexane, causing a dark brown solution to be formed. Stirring was continued for 2 h at 0 °C, followed by dropwise addition of 3-fluorobenzyl bromide (25 g, 132.3 mmol) in THF (50 ml). The reaction mixture was gradually warmed to room temperature and after 3 h (TLC monitoring), it was quenched with 10% HCl (20 ml). The mixture was poured into ice-water (200 ml), extracted with CHCl<sub>3</sub> (2x150 ml), the organic layers were collected, washed with brine (150 ml), chromatographed on silica gel using CH<sub>2</sub>Cl<sub>2</sub> and EtOAc successively as eluent, furnishing 19.5 g of a slightly yellow oil. *R<sub>f</sub>* = 0.37 (silica gel, EtOAc:acetone 1:1)

(1S,5R,6R,7R)-6-Formyl-7-(4-phenyl benzoyloxy)-2-oxabicyclo[(3.3.0)]octane-3-one  
19

To a solution of the alcohol **18** (19.0 g, 53.9 mmol) in DME (100 ml), cooled to 18 °C, was added dicyclohexylcarbodiimide (DCC) (33.3, 161.8 mmol), DMSO (38.2 ml) and phosphoric acid (1.43 ml, 21.28 mmol). The temperature of the reaction mixture was kept below 25 °C for 30 min. The reaction mixture was stirred at room temperature for additional 2 hours (TLC monitoring), and the precipitate was removed

by filtration and washed with ether (2x50 ml). The combined organic layer was washed with water (50 ml) and brine (2x50 ml), the aqueous solution was extracted with ether (100 ml), the organic layers were collected and dried over sodium sulfate, filtered, and used directly for the next step. TLC  $R_f$  = 0.37 (silica gel, EtOAc:toluene 2:1).

(1S,5R,6R,7R)-6-(3-Oxo-5-(3-fluorophenyl)-1-E-pentenyl)-7-(4-phenyl benzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (20)

To a stirred suspension of NaH (1.9 g, 65.1 mmol), prewashed with n-pentane, in DME (130 ml) under nitrogen, was added dropwise dimethyl-2-oxo-4-(3-fluorophenyl) butylphosphonate (Wadsworth, Jr., W. S., et al. 1961) (19.3 g, 70.5 mmol), in DME (100 ml) and stirred vigorously for 1 h at room temperature. The mixture was then cooled to -10 °C and a solution of the crude aldehyde **19** was added dropwise. After 30 min. at 0 °C and 2 h at room temperature (TLC monitoring), the reaction mixture was neutralized with acetic acid, the solvent was removed in vacuo and the residue was dissolved in EtOAc (200 ml), and washed with water (50 ml) and brine (50 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated in vacuo. The residue was stirred with ether (100 ml), the resulting white precipitate was filtered and washed with cold ether, giving a white crystalline substance yield (17 g, 58.5%)  $R_f$  = 0.56 (silica gel, ethyl acetate : toluene 2:1)

(1S,5R,6R,7R)-6-(3S-3-Hydroxy-5-(3-fluorophenyl)-1-pentenyl)-7-(4-phenyl benzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (21)

To a stirred solution of the enone **20** (17.1 g, 34.3 mmol) in THF (20 ml) and cerium chloride ( $\text{CeCl}_3 \cdot 7 \text{H}_2\text{O}$ ) (3.8 g, 10.3 mmol) in THF:ether 1:2 (60 ml) cooled to -20 °C under nitrogen was added sodium borohydride (0.8 g, 20.57 mmol) in small portions. The reaction mixture was stirred for 2 h (TLC monitoring). The temperature was raised to  $\pm 0$  °C, then quenched by adding water (20 ml) and an aqueous solution of 10% HCl to pH 4 and extracted with EtOAc (50 ml). The organic layer was separated and washed with brine, dried on anhydrous sodium sulfate, concentrated in vacuo and

chromatographed twice on silica gel using toluene:EtOAc 2:1 and 1:1 successively as eluent, furnishing **4** (5 g) as a white crystalline product  $R_f = 0.32$  (silica gel, EtOAc:toluene 2:1).

(1S,5R,6R,7R)-6-{3R-3-Hydroxy-5-(3-fluorophenyl)-1-pentyl}-7-(4-phenylbenzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (22)

To a suspension of 10% Pd/C (0.1 g) in sodium nitrite (3.6 ml, 1.8 mmol) and ethanol (15 ml) was added a solution of **21** (3 g, 6.0 mmol) in ethanol (6.0 ml). The mixture was stirred under hydrogen atmosphere for 6 h (TLC monitoring), and quenched with 1M solution of HCl. The catalyst was removed by filtration through a celite pad, washed with ethanol abs. (15 ml). The solvent was removed in vacuo. The resulting oil was dissolved in EtOAc (100 ml), and washed with brine 15% (30 ml). The water phase was washed with EtOAc (40 ml). The combined organic extracts were dried over sodium sulfate and filtered. The solvent was removed in vacuo. The residue was chromatographed on silica gel using EtOAc as eluent, which gave **5** (2.94 g),  $R_f = 0.25$  (silica gel, EtOAc).

(1S,5R,6R,7R)-6-{3R-3-Hydroxy-5-(3-fluorophenyl)-1-pentyl}-7-R-hydroxy-2-oxabicyclo[3.3.0]octane-3-one (23)

To a solution of the lactone **22** (2.8 g, 5.65 mmol) in methanol (15 ml) was added potassium carbonate (0.47 g, 3.3 mmol) and the mixture was stirred at ambient temperature for 6 h (TLC monitoring). The mixture was neutralised with 10% aqueous solution of HCl and extracted with EtOAc (2x30 ml). The organic phase was dried on anhydrous sodium sulfate and evaporated to dryness. The crude product was chromatographed on silica gel using EtOAc:acetone 1:1 as eluent. The title compound **23** was obtained as a white crystalline product; yield 1.6 g,  $R_f = 0.17$  (silica gel, EtOAc);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.2-1.4 (m, 1H), 1.54 (m, 3H), 1.8 (m, 3H), 2.1 (m, 1H), 2.2 (m, 1H), 2.3 (m, 1H), 2.6 (m, 2H), 2.67 (m, 1H), 2.8 (m, 2H), 3.60 (m,  $\text{CH}_2\text{CHOHCH}_2$ ), 4.0 (m,  $\text{CHOH}$ ) 4.92 (m,  $\text{CHOC=O}$ ), 6.8-7.0 (m, 3H), 7.28 (m, 1H).

(1S,5R,6R,7R)-6-{3R-3-t-butyl dimethylsilyloxy-5-(3-fluorophenyl)-1-pentyl}-7-R-t-butyl dimethylsilyloxy-2-oxabicyclo[3.3.0]octane-3-one (24)

*t*-Butyldimethylsilyl chloride (2.3 g, 14.9 mmol) was added in one portion to a solution of the diol **23**, triethyl amine (2.1 ml, 14.8 mmol) and 4-dimethylamino pyridine (0.06 g, 0.1 mmol) in dichloromethane (20 ml) with vigorous stirring at room temperature for 24 h, and the reaction mixture was concentrated in vacuo. The crude product was dissolved in ethyl acetate (50 ml), washed with water (20 ml) and 5% aqueous solution of sodium hydrogen carbonate (20 ml). The organic phase was dried on sodium sulfate, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using dichloromethane as eluent to give 3 g of the product as oil.  $R_f$  = 0.68 (silica gel, ether)

(1S,5R,6R,7R)-6-{3R-3-*t*-butyl dimethylsilyloxy-5-(3-fluorophenyl)-1-pentyl}-7-*R*-*t*-butyl dimethylsilyloxy-2-oxabicyclo[3.3.0]octane-3-ol (**25**)

A solution of diisobutylaluminium hydride (DIBAL) (1.1 g, 7.43 mmol) in dry toluene (5.3 ml) was added dropwise to a stirred solution of the lactone **24** (2.7 g, 4.95 mmol) in dry THF (30 ml) at -72/-80 °C. After 1 h (TLC monitoring), the reaction mixture was quenched with methanol (5 ml) and was warmed to room temperature, and added water (50 ml), 10% aqueous solution of HCl (50 ml), extracted with EtOAc (2x50 ml). The organic layer was dried with sodium sulfate, filtered, the solvent was removed in vacuo, and the residue was chromatographed on silica gel using EtOAc and EtOAc:acetone 1:1, respectively, as eluent, to give a yellow oil product (2.7 g),  $R_f$  = 0.85 (silica gel, ethyl acetate 1:1).

13,14-Dihydro-11,15-di-*t*-butyldimethyl silyloxy-17-(3-fluorophenyl)-18,19,20-trinor-PGF<sub>2α</sub> (**26**)

To a stirred suspension of 4-carboxybutyl triphenyl phosphonium bromide (8.78 g, 19.82 mmol) in THF (50 ml) under nitrogen at 0-5 °C was added potassium *t*-butoxide (3.89 g, 34.6 mmol), and the mixture stirred for 30 min. at room temperature. To the resultant red orange solution of ylide at -15/-10 °C was added the lactol **25** (2.7 g, 4.95 mmol) in THF (10 ml), and the mixture was stirred for 3-4 h (TLC monitoring). The reaction mixture was diluted with water (30 ml) and washed with ether (4x40 ml). The water layer was acidified with 5% aqueous solution of citric acid to pH 4 and extracted with EtOAc (2x50 ml). The organic phase was washed

with brine (30 ml), dried on sodium sulfate, and filtered. The solvent was removed in vacuo, and the slurry **26** was used directly without isolation for the next step.

13,14-Dihydro-11,15-di-t-butyl dimethyl silyloxy 17-(3-fluorophenyl)-18,19,20-trinor PGF<sub>2α</sub> isopropyl ester (27)

DBU (5.28 g, 34.7 mmol) was added dropwise to a stirred solution of the crude product **26** (3.16 g, 4.96 mmol) in acetone (20 ml) at 0 °C. The mixture was allowed to warm to room temperature, and isopropyl iodide (5.05 g, 29.7 mmol) was added dropwise. After 4 h (TLC monitoring), the mixture was diluted with EtOAc (100 ml), washed with brine (30 ml), citric acid 3% (2x25 ml) and sodium hydrogen carbonate 5% (2x25 ml) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel using ether:petroleum ether 1:2 as eluent. This afforded a colourless oil, yield 1.7 g,  $R_f = 0.43$  (silica gel, ether : petroleum ether 1:2) <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.1 (m, 9H), 0.9 (m, 16H), 1.2 (m, 9H), 1.6-1.8 (mm, 10H), 2.12 (m, 2H), 2.22-2.33 (m, 2H), 2.6-2.9 (dm, 2H), 3.65 (m, CH<sub>2</sub>CHOHCH<sub>2</sub>), 3.94 (m, CH<sub>2</sub>CHOH), 4.16 (m, CH<sub>2</sub>CHOH), 5.0 (sept. 1H), 5.38 (m, db), 5.47 (m, db), 6.8-7.0 (dm, Ar, 3H), 7.2 (m, Ar, 1H).

13,14-Dihydro-11,15-di-t-butyl dimethyl silyloxy 17-(3-fluorophenyl)-18,19,20-trinor PGE<sub>2</sub> isopropyl ester (28)

Pyridinium dichlorochromate (2.43 g, 11.25 mmol) on aluminum oxide (20 g) was added in small portions to a solution of **27** (1.7 g, 2.5 mmol) in dichloromethane (30 ml) and the mixture was stirred at room temperature (TLC monitoring), filtered, and the precipitate was washed with ether: ethyl acetate 2:1. The solvent was removed in vacuo. The residue was diluted with ether (100 ml) and washed with water (30 ml), 5% aqueous solution of NaHCO<sub>3</sub> (3x20 ml), the organic phase was separated and dried over sodium sulfate, and evaporated in vacuo to give **28** (1.3 g), as an oil.  $R_f = 0.72$  (silica gel, ethyl acetate).

13,14-Dihydro-17-(3-fluorophenyl)-18,19,20-trinor PGE<sub>2</sub> isopropyl ester (7)



Hydrogen fluoride 15% (12 ml) was added to a solution of 28 (314 mg) in acetonitrile. The mixture was stirred at room temperature for 4 h (TLC monitoring). The reaction mixture was diluted with ethyl acetate (100 ml) and washed with water (3x20 ml), dried and evaporated in vacuo. The residue was chromatographed on silica gel using ethyl acetate as eluent, which gave 7 (64 mg) as an oil,  $R_f = 0.43$  (silica gel, ethyl acetate).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.2 (d, 6H), 1.6-1.8 (m, 6H), 1.8 (m, 2H), 2.12 (m, 2H), 2.2-2.3 (m, 2H), 2.6-2.8 (dm, 2H), 3.6 (m,  $\text{CH}_2\text{CHOHCH}_2$ ), 4.16 (m,  $\text{CH}_2\text{CHOH}$ ), 5.0 (sept. 1H), 5.38 (m, db), 5.47 (m, db), 6.8-7.0 (dm, Ar, 3H), 7.2 (m, Ar, 1H).

### Pharmacology

#### Intraocular pressure reducing effect of the test compounds in cats and monkeys.

The compounds were tested for intraocular pressure reducing effect in animal models. The intraocular pressure was measured with a calibrated pneumotonometer. European domestic cats and cynomolgus monkeys were used as experimental animals. The cornea was anaesthetized with oxibuprocain before the measurement. The reductions in intraocular pressure after topical treatment with the  $\text{PGF}_{2\beta}$  isopropyl ester (2), 17-phenyl-18,19,20-trinor- $\text{PGE}_2$ -isopropyl ester (4), 15RS-16,16-trimethylene-methyl ester (6) and 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor- $\text{PGE}_2$ -isopropyl ester (7) are demonstrated in Tables IV and V.

**Table IV.** Intraocular pressure reducing effect of 1-10  $\mu$ g of the test compounds, with effect on the EP<sub>1</sub> prostanoid receptor, in cats. The control eye received the vehicle only. (n= 5-6; Mean $\pm$ SEM).

Prostaglandin/ Eye	Baseline pressure (mmHg)	Pressure 3 h after treatment (mmHg)
<u>2</u>		
Experimental eye	24.2 $\pm$ 2.3	15.1 $\pm$ 2.8 *
Control eye	24.5 $\pm$ 2.7	22.5 $\pm$ 3.4
<u>4</u>		
Experimental eye	22.0 $\pm$ 1.7	14.2 $\pm$ 1.7 *
Control eye	21.5 $\pm$ 1.7	18.7 $\pm$ 1.9
<u>6</u>		
Experimental eye	19.2 $\pm$ 1.7	9.5 $\pm$ 0.5 *
Control eye	19.3 $\pm$ 1.7	17.0 $\pm$ 1.3
<u>7</u>		
Experimental eye	20.4 $\pm$ 2.0	14.2 $\pm$ 0.9 *
Control eye	20.6 $\pm$ 1.8	18.4 $\pm$ 1.5

\* p<0.01 (matched pair t-test between eyes)

**Table V.** Intraocular pressure reducing effect of the test compounds, with effect on the EP<sub>1</sub> receptor, in monkeys. The dose of PGF<sub>2 $\beta$</sub> -isopropyl ester was 30  $\mu$ g, while that of 17-phenyl-18,19,20-trinor-PGE<sub>2</sub>-isopropyl ester, and 15RS-16,16-trimethylene-PGE<sub>2</sub>-isopropyl ester was 3  $\mu$ g. The control eye received the vehicle only (n=6; Mean $\pm$ SEM).

Prostaglandin/ Eye	Baseline pressure (mmHg)	Pressure 4 h after treatment (mmHg)
<u>2</u>		
Experimental eye	17.8 $\pm$ 1.4	14.1 $\pm$ 1.8 *
Control eye	16.9 $\pm$ 1.2	17.5 $\pm$ 2.0
<u>4</u>		
Experimental eye	14.1 $\pm$ 1.1	9.9 $\pm$ 0.9 *
Control eye	13.9 $\pm$ 1.0	11.5 $\pm$ 0.8
<u>6</u>		
Experimental eye	20.9 $\pm$ 1.6	15.3 $\pm$ 2.4 *
Control eye	21.3 $\pm$ 1.5	19.0 $\pm$ 1.5

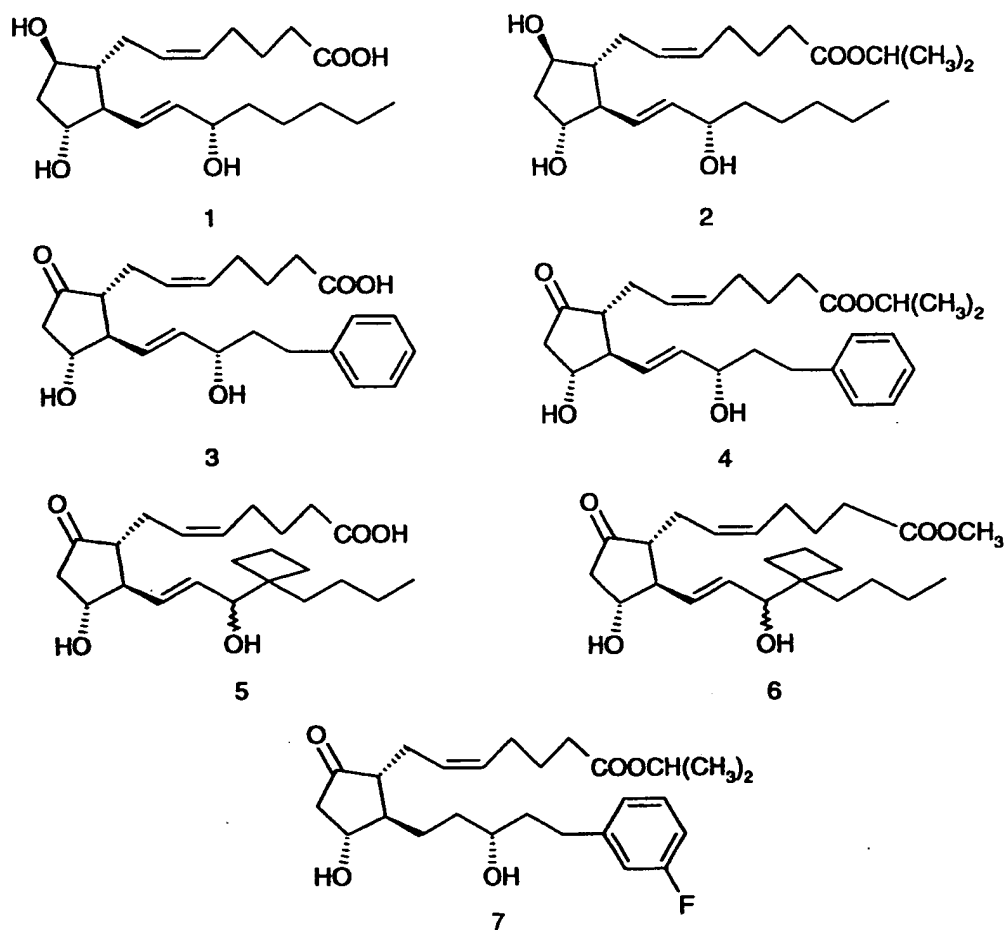
\* p<0.05 (match paired t-test between the eyes)

It can be seen that both in cats and monkeys all the prostaglandin analogues with preference for the EP<sub>1</sub> receptor significantly reduced the intraocular pressure.

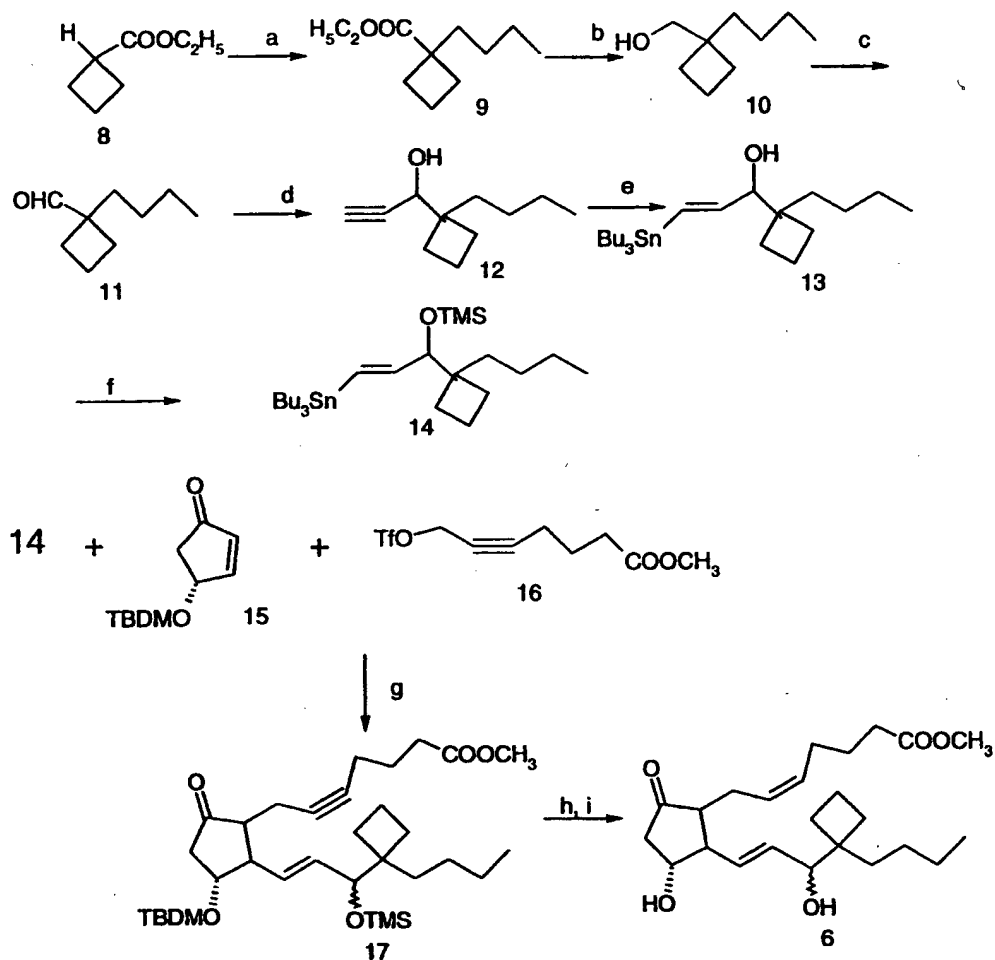
Accordingly, the present invention discloses that compounds with selective stimulatory effect on EP<sub>1</sub> receptors reduce the intraocular pressure, and that such compounds cannot have any melanogenic effect, or at least have significantly reduced effect in the eye since the pigment producing cells, the melanocytes, lack the EP<sub>1</sub>

receptor in man. Thus, the common side-effect of increased iridial pigmentation can be avoided during chronic therapy with prostaglandins selective for EP<sub>1</sub> receptors.

Scheme 1



Scheme 2



## Reagents

a. N-isopropylcyclohexyl amine / THF, n-BuLi, ethylcyclobutanecarboxylate/ DMSO

b. DIBAL-H, / toluene

c. DCC/ DME, DMSO, H<sub>3</sub>PO<sub>4</sub>,

d. Lithium-acetylide-ethylene diamine, DMSO

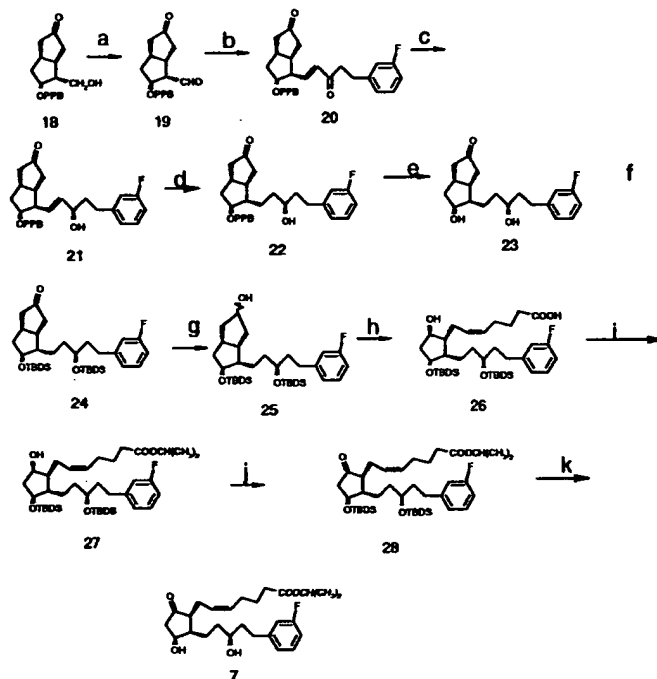
e. tributyltin hydride, AIBN

f. Trimethylsilyl chloride (TMSCl), imidazole/ DMF

g. Li<sub>2</sub>CuCN(CH<sub>3</sub>)<sub>2</sub>, TMSCl, triethylamine, 4-*t*-butyl-dimethylsilyloxy-2-cyclopentenone, 1-tributyltin-4,4,-trimethylene-3-trimethylsilyloxy-1-octene, methyl-2-yn-8-octanoateh. Pd-BaSO<sub>4</sub>, quinoline,

i. HF/THF

Scheme 3



## Reagents

- a. DCC, DMSO, H<sub>2</sub>SO<sub>4</sub>, DME, H<sub>3</sub>PO<sub>4</sub>
- b. NaH, dimethyl-2-oxo-4-(3-fluorophenyl)-butylphosphonate
- c. NaBH<sub>4</sub>, CeCl<sub>3</sub>·7H<sub>2</sub>O/THF
- d. Pd/C, NaNO<sub>2</sub>/THF
- e. K<sub>2</sub>CO<sub>3</sub>/Methanol
- f. TBDMS, TEA, 4-dimethylamino pyridine/dichloromethane
- g. DIBAL-H/THF
- h. 4-carboxybutyl triphenyl phosphonium bromide  
potassium t-butoxide, THF
- i. DBU, isopropyl iodide/acetone
- j. pyridinium chlorochromate, aluminium oxide/dichloromethane
- k. HF/acetonitrile

### References

Bill, A. (1975). Blood circulation and fluid dynamics in the eye. *Physiol. Rev.* 55; 383-417.

Coleman, R.A., Smith, W.L. and Narumiya, S. (1994). VIII. International Union of Pharmacology classification of prostanoid receptors: Properties, distribution and structure of the receptors and their subtypes. *Pharmacol. Rev.* 46; 205-229.

Crawford, K., and Kaufman, P. (1987). Pilocarpine antagonizes  $\text{PGF}_{2\alpha}$ -induced ocular hypotension in monkeys. *Arch. Ophthalmol.* 105; 1112-1116.

Ernhardt, P.W, Owens, A. H. (1987) Facile Formation of Quaternary azetidinium compounds During Triflation of Dialkylaminopropanols. *Synth. Commun.* 17, 469-475.

Caldwell, A., G., Harris, C. J., Stepny, R., Whittaker, N. (1979). Hydantoin Prostaglandin analogues, Potent and Selective Inhibitors of Platelet Aggregation. *J. C. S. Chem. Commun.* 561.

Skotnicki, S., Schaub, E., Weiss, J. (1977). Prostaglandins and congeners. 14. Synthesis and Bronchodilator Activity of dl-16,16-trimethyleneprostaglandins. *J. Med. Chem.* 20, 1042.

Hu, D-N. et al. (1993). *Investigative Ophthalmology and Visual Science* 34; 2210-2219.

Nilsson, S.F.E., Samuelsson, M., Bill, A., and Stjernschantz, J. (1989). Increased uveoscleral outflow as a possible mechanism of ocular hypotension caused by prostaglandin  $\text{F}_{2\alpha}$ -isopropyl ester in the cynomolgus monkey. *Exp. Eye Res.* 48; 707-716.

Stjernschantz, J., Selén, G., Sjöquist, B., and Resul, B. (1995). Preclinical pharmacology of latanoprost. *Advances in Prostaglandin, Thromboxane and Leukotriene Research* 23; 513-518.

Stjernschantz, J. and Alm, A. (1996). Latanoprost as a new horizon in the medical management of glaucoma. *Current Opinion in Ophthalmology* 7; 2: 11-17.

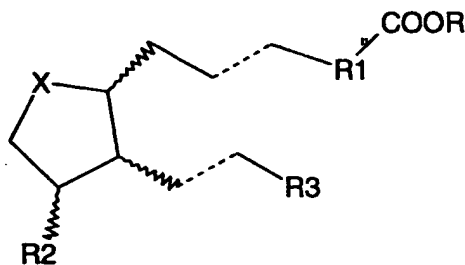
Toris, C., Camras, C.B., and Yablonski, M.E. (1993). Effects of PhXA41, a new prostaglandin F<sub>2α</sub> analogue, on aqueous humor dynamics in human eyes. *Ophthalmology* 10; 1297-1304.

Wodswarth, Jr., W. S., Emmon, W. D. (1961). The Utility of Phosphonate Carbanions in Olefin Synthesis. *J. Am. Chem. Soc.* 83, 1733.



## CLAIMS

1. A composition for the treatment of glaucoma and ocular hypertension comprising a therapeutically active and physiologically acceptable amount of a prostaglandin analogue which is a selective agonist for EP<sub>1</sub> prostanoid receptors, or a pharmaceutically acceptable salt or ester thereof.
2. The composition according to claim 1, wherein the prostaglandin analogue is derived from PGF or PGE type prostaglandins.
3. The composition according to claim 1 or 2, wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the  $\alpha$  or  $\beta$  configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably C<sub>1-10</sub> alkyl, cycloalkyl, preferably C<sub>3-8</sub> cycloalkyl, aryl, arylalkyl, preferably aryl-C<sub>2-5</sub> alkyl, or heteroaryl;

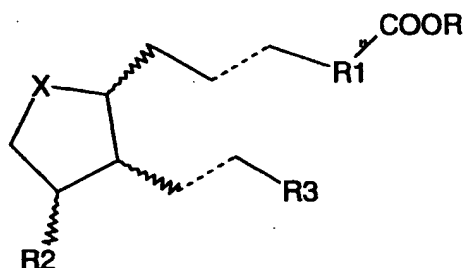
R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by a heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably C<sub>3-7</sub> cycloalkyl, cycloalkenyl, preferably C<sub>3-7</sub> cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR<sub>4</sub>, where R<sub>4</sub> is a straight or branched chain saturated or unsaturated alkyl group, preferably C<sub>1-10</sub> alkyl, especially C<sub>1-6</sub> alkyl, or a cycloalkyl, preferably C<sub>3-8</sub> cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C<sub>1-5</sub> alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably C<sub>3-8</sub> cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with C<sub>1-3</sub> alkyl, C<sub>1-3</sub> alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

4. The composition according to claim 1, 2 or 3, wherein the prostaglandin analogue is 15(R,S)-16,16-trimethylene-PGE<sub>2</sub> or an alkyl ester thereof.
5. The composition according to claim 1, 2 or 3 wherein the prostaglandin analogue is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE<sub>2</sub> or an alkyl ester thereof.
6. A method of treating glaucoma or ocular hypertension in a subject's eye, which method comprises contacting the surface of the eye with an effective intraocular pressure reducing amount of a therapeutically active and physiologically acceptable prostaglandin analogue which is a selective agonist for EP<sub>1</sub> prostanoid receptors, or a pharmaceutically acceptable salt or ester thereof.
7. The method according to claim 6, wherein the prostaglandin analogue is derived from PGF or PGE prostaglandins.
8. The method according to claim 6 or 7, wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the  $\alpha$  or  $\beta$  configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably  $C_{1-10}$  alkyl, cycloalkyl, preferably  $C_{3-8}$  cycloalkyl, aryl, arylalkyl, preferably aryl- $C_{2-5}$  alkyl, or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by a heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably  $C_{3-7}$  cycloalkyl, cycloalkenyl, preferably  $C_{3-7}$  cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, preferably  $C_{1-10}$  alkyl, especially  $C_{1-6}$  alkyl, or a cycloalkyl, preferably  $C_{3-8}$  cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from  $C_{1-5}$  alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably  $C_{3-8}$  cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with  $C_{1-3}$  alkyl,  $C_{1-3}$  alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

9. The composition according to claim 6, 7 or 8, wherein the prostaglandin analogue is 15(R,S)-16,16-trimethylene-PGE<sub>2</sub> or an alkyl ester thereof.

10. The composition according to claim 6, 7 or 8 wherein the prostaglandin analogue is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE<sub>2</sub> or an alkyl ester thereof.
11. The method according to any one of claims 6-10, wherein a therapeutically active and physiologically acceptable composition containing said prostaglandin analogue is administered topically on the eye 1-3 times daily.
12. Use of a prostaglandin analogue which is a selective agonist for EP<sub>1</sub> prostanoid receptors as defined in any one of claims 1 to 4 for the preparation of a medicament for treatment of glaucoma and ocular hypertension.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01368

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/557

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9408585 A1 (ALCON LABORATORIES, INC.), 28 April 1994 (28.04.94) --	1-3,12
X	Journal of Lipid Mediators, Volume 6, 1993, David F. Woodward et al, "Intraocular pressure effects of selective prostanoid receptor agonists involve different receptor subtypes according to radioligand binding studies" page 545 - page 553 --	1-3,12
A	The Journal of Biological Chemistry, Volume 268, No 27, Sept 1993, Akiko Watabe et al, "Cloning and Expression of cDNA for a Mouse EP1 Subtype of Prostaglandin E Receptor", page 20175 - page 20178 --	12

☒ Further documents are listed in the continuation of Box C.
 ☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document but published on or after the international filing date	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search  12 October 1998	Date of mailing of the international search report  01 - 11 - 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer  Göran Karlsson Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01368

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Natural product reports, Volume 7, No 5, 1990, D. E. Bays et al, "Inhibitors of Gastric Acid Secretion", page 409 - page 445, see page 436  ---	12
X	US 4132738 A (HAROLD C. KLUENDER ET AL), 2 January 1979 (02.01.79)  -- -----	1-4

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01368

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 6-8, 11  
because they relate to subject matter not required to be searched by this Authority, namely:  
A method for treatment of the human or animal body by therapy, see rule 39.1.
2. ☒ Claims Nos.: 12  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
The expression "a selective agonist for EP1 prostanoid receptors" in claim 12 is indefinite. According to PCT Article 6, the claims shall be clear and concise. Claim 12 has therefore not been fully searched.
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

27/07/98

International application No.

PCT/SE 98/01368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9408585 A1	28/04/94	AT 153855 T	15/06/97
		AU 674038 B	05/12/96
		AU 5328694 A	09/05/94
		DE 69311361 D,T	08/01/98
		EP 0664707 A,B	02/08/95
		ES 2105333 T	16/10/97
		JP 8502485 T	19/03/96
		US 5480900 A	02/01/96
		US 5605922 A	25/02/97
<hr/>			
US 4132738 A	02/01/79	AU 515772 B	30/04/81
		AU 4449379 A	30/08/79
		CA 1191132 A	30/07/85
		DE 2902699 A,B,C	30/08/79
		FR 2430403 A,B	01/02/80
		FR 2473509 A,B	17/07/81
		GB 2014989 A,B	05/09/79
		GB 2099814 A,B	15/12/82
		JP 1194012 C	12/03/84
		JP 54115351 A	07/09/79
		JP 58026910 B	06/06/83
		SE 441673 B,C	28/10/85
		SE 445109 B,C	02/06/86
		SE 453830 B,C	07/03/88
		SE 453990 B,C	21/03/88
		SE 7813385 A	24/08/79
		SE 8303910 A	08/07/83
		SE 8303911 A	08/07/83
		SE 8303912 A	08/07/83
		US 4275224 A	23/06/81
		US 4331688 A	25/05/82
		US 4415592 A	15/11/83
		US 4742080 A	03/05/88
		US 4833157 A	23/05/89
<hr/>			